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Mutagenicity of bromate: Implications for cancer risk assessment

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Abstract

Bromate (BrO_3^-) is a rodent carcinogen that is formed as a drinking water ozone disinfection by-product and also used in some food and consumer products. Therefore, bromate is subject to assessment for its risk to humans. Because the selection of an appropriate model for conducting quantitative cancer risk assessment is based upon an understanding of the chemical's mode-of-action, it is necessary to determine whether the chemical is a mutagenic carcinogen. We present a review of the available information concerning the weight-of-the-evidence that bromate is a mutagenic carcinogen. The evidence indicates that bromate is mutagenic and that this activity is mediated by the formation of oxidative damage to the DNA, thus resulting in chromosomal damage. Not only does bromate induce genetic damage in vitro, it is also demonstrated to induce mutations in the kidney of exposed rats. This is significant because the rat kidney is one of the target tissues for tumor induction. While it is clear that bromate can cause damage in the target tissue, it is not clear whether bromate is a mutagenic carcinogen, that is, whether the observed tumors result from a mutagenic mode-of-action. Further research is needed to clarify bromate's mode-of-action. However, in the absence of additional information, it is reasonable, based on an extensive database, to assume that bromate induces tumors via oxidative damage that causes chromosomal breakage.

Keywords: Potassium bromate; Sodium bromate; Mutagenicity; Genotoxicity; Risk assessment

1. Introduction

Bromate (BrO_3^-) is a byproduct of ozone disinfection of drinking water (Fiessinger et al., 1985; Kurokawa et al., 1982). Furthermore, because of its oxidizing properties, potassium bromate ($KBrO_3$) is widely used as a part of the bread-making process for the maturation of flour and is, therefore, a food additive. It is also used in the production of fish paste and fermented beverages. In addition, $KBrO_3$ is used in cold-wave hair lotion (IARC, 1986). The International Agency for Research on Cancer (IARC) has classified $KBrO_3$ as a 2B (a possible human) carcinogen (IARC, 1987) based on sufficient evidence that $KBrO_3$ induces cancer in experimental animals (IARC, 1986).

$KBrO_3$ induces renal cell tumors, mesotheliomas of the peritoneum and follicular cell tumors of the thyroid in rats and renal cell tumors in mice following long-term oral administration in drinking water (DeAngelo et al., 1998; Kurokawa et al., 1986, 1990). According to experiments aimed at elucidating the mode of carcinogenic action, Kurokawa et al. (1990) suggested that $KBrO_3$ was a complete carcinogen, possessing both initiating and promoting activities for rat renal tumorigenesis. This hypothesis has been supported by additional research. $KBrO_3$-induced chromosome

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aberration, micronuclei, gene mutations and loss of heterozygosity (LOH). This data will be outlined in our review. Also $KBrO_3$ enhanced formation of *N*-ethyl-*N*-hydroxyethyl-nitrosamine initiated renal tumors in rats due to cellular proliferation produced by $KBrO_3$-induced oxidative stress (McLaren et al., 1994; Umemura et al., 1995).

Any new assessment of human cancer risk from exposure to bromate will include an assessment of the mode-of-action by which it induces cancer. In the present paper, we review the evidence that bromate can induce genetic damage with an emphasis on its ability to induce mutation and therefore the possibility that it is a "mutagenic" carcinogen. For this assessment, we follow the general strategy for conducting a weight-of-the-evidence assessment for mutagenic potential as outlined previously (Moore and Harrington-Brock, 2000). Briefly, this involves considering the evidence that bromate can induce primary DNA damage in vitro and in vivo; mutations in vitro; the types of the induced genetic damage. It also includes an evaluation as to whether bromate induces mutations in vivo and most importantly whether mutational damage has been demonstrated to occur in the target tissue(s).

2. Evidence that bromate induces primary DNA damage either in vitro or in vivo

2.1. DNA strand breakage assays

The oxidative DNA damage induced by $KBrO_3$ in cultured mammalian cells and in a cell-free system was characterized by an alkaline elution assay and glutathione (GSH). Under cell-free conditions, no modifications were induced by $KBrO_3$ alone, but extensive DNA damage was observed in the presence of (GSH). In L1210 mouse leukemia cells and LLC-PK1 porcine kidney cells, $KBrO_3$ alone gave rise to a DNA damage profile similar to that observed after treatment of cell-free DNA with $KBrO_3$ plus GSH (Ballmaier and Epe, 1995).

Using the Comet assay in V79 Chinese hamster cells exposed to $KBrO_3$, Speit et al. (1999) found that potassium bromate treatment significantly increased levels of 8-oxodeoxyguanosine (8-OH-G) by HPLC analysis thus providing direct evidence that the induced DNA damage is caused by the induction of 8-OH-G. The induction of DNA breakage using the Comet assay was confirmed in CHO-K1 cells (Poul et al., 2004), in primary rat kidney cells (Robbiano et al., 1999) and in CHO-AS52 cells (Plewa et al., 2002). In addition, Robbiano et al. (1999) demonstrated that $KBrO_3$ can cause DNA strand breakage in vivo. Their study used male Sprague–Dawley rats that were treated per os and subjected to unilateral nephrectomy.

2.2. Chromosome aberration

It is clear that $KBrO_3$ can induce chromosome aberrations in cultured cells. Chinese hamster lung (CHL) cells treated with 0.0625–0.25 mg/ml $KBrO_3$ without metabolic activation had significantly higher rates of chromosome aberrations than the controls (Ishidate et al., 1984, 1981; Ishidate and Yoshikawa, 1980; Ishidate, 1987). In addition Chinese hamster DON-6 cells treated with $KBrO_3$ showed a positive response for aberration induction (Sasaki et al., 1980). The main aberration types observed were chromatid type breaks and chromatid exchanges. The clastogenic activity of $KBrO_3$ was considered to be relatively strong (Ishidate, 1987). A similar result also was found in V79 cells exposed to $KBrO_3$ (Speit et al., 1999). A slight increase in the frequency of aberrations was seen at 5 and 10 mM and a strong induction at 20 mM. As in the previously mentioned studies, the majority of the observed chromosome aberrations were chromatid breaks and chromatid exchanges. Kawachi et al. (1980) reported that $KBrO_3$ induced chromosome aberrations in rats.

2.3. Micronucleus analysis

There is evidence that $KBrO_3$ can induce micronuclei in vitro. Speit et al. (1999), using V79 Chinese hamster cells demonstrated the induction of micronucleus following 1 h $KBrO_3$ treatment. The percentage of micronucleated cells was about 1, 3, 8 and 19% at concentration 0, 2.5, 5 and 10 mM, respectively. Similar results were obtained by using CHO-K1 cells (Poul et al., 2004).

It is clear that micronuclei can be induced following in vivo exposure to $KBrO_3$. Studies have been performed using several different mouse strains with similar results. Micronucleated polychromatic erythrocytes were induced in male ddY mice in a dose-dependent manner when $KBrO_3$ was administered at doses higher than 25 and 100 mg/kg body weight by intraperitoneal (i.p.) and oral routes (Hayashi et al., 1988). No sex differences were evident in ddY mice for the induction of micronuclei after a single i.p. administration of $KBrO_3$. Male MS/Ae and CD-1 mice were treated with i.p. doses of 18.8, 37.5, 75 and 150 mg/kg and oral doses of 37.5, 75, 150 and 300 mg/kg $KBrO_3$. The sampling time was 24 h for both mouse strains. $KBrO_3$ induced a dose-dependent increase in micronucleated polychromatic erythrocytes after both routes of administration